SURVIVAL OF SURFACE-INOCULATED *LISTERIA MONOCYTOGENES* ON COMMERCIALLY AVAILABLE FRANKFURTERS FOLLOWING GAMMA IRRADIATION[1]

C.H. SOMMERS[2] and D.W. THAYER

U.S. Department of Agriculture
Agricultural Research Service
Eastern Regional Research Center
600 East Mermaid Lane
Wyndmoor, PA 19038

Accepted for Publication April 25, 2000

528900

ABSTRACT

Frankfurter is a generic term for a cured and cooked sausage, which may consist of almost any meat type and include a wide variety of nonmeat fillers and additives. When several "brands" or types of commercially available frankfurters were surface-inoculated with Listeria monocytogenes *and vacuum-packed, gamma radiation D-values ranged from 0.49 kGy to 0.71 kGy, with an average D-value of 0.61 kGy. Differences in gamma radiation D-value were observed for nine of twenty one pair-wise comparisons (α = 0.01) as determined by analysis of covariance. Therefore, frankfurter formulation may affect radiation D-values for surface inoculated* L. monocytogenes. *If low dose gamma irradiation, cold pasteurization, were to be used for control of* L. monocytogenes *on frankfurters, gamma radiation dosage should be based on individual product formulation.*

INTRODUCTION

Listeria monocytogenes is a food-borne pathogen capable of growth at refrigerated temperatures and in high salt environments. Such growth produces no

[1]Mention of brand or firm names does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

[2]Corresponding author. Telephone: (215) 836-3754; FAX: (215) 233-6445;
E-mail: csommers@arserrc.gov

Journal of Food Safety **20** (2000) 127-137. *All Rights Reserved.*
©*Copyright 2000 by Food & Nutrition Press, Inc., Trumbull, Connecticut.*

apparent signs of spoilage in food products (Smith 1996; Glass and Doyle 1989; Hudson and Mott 1993; Hudson *et al.* 1994; Manu-Tawiah *et al.* 1993). Because of the high mortality rate associated with listeriosis, approximately 30% of immuno-compromised individuals, *L. monocytogenes* was classified as a zero tolerance organism in precooked ready-to-eat meat products (Wehr 1987; USDA 1989).

Post-process contamination of ready-to-eat meat products with *L. monocytogenes* is well documented (Wang and Muriana 1994; Wenger *et al.* 1990; Farber *et al.* 1990; Kerr *et al.* 1990; Grau and Vanderlinde 1992). In 1998 approximately 2.5% of ready-to-eat meat products tested by the Food Safety Inspection Service were positive for *L. monocytogenes* (Nickelson and Schmidt 1999). A number of food-borne illness outbreaks and recalls of ready-to-eat meat products, including frankfurters, have been attributed to *L. monocytogenes* (Schwartz *et al.* 1988; Barnes *et al.* 1989; Centers for Disease Control 1989; Nickelson and Schmidt 1999).

Low dose gamma irradiation (cold pasteurization) effectively eliminates *L. monocytogenes* from raw, cured, and cooked meat products. (Monk *et al.* 1994; Fu *et al.* 1995; Patterson, 1989; Patterson *et al.* 1993; Thayer *et al.* 1998; Thayer and Boyd 1995). Our objectives were to answer the following questions: (1) What are the gamma radiation D-values for *L. monocytogenes* obtained from surface inoculated commercially available frankfurters? (2) Are the gamma radiation D-values consistent with those obtained for other meat products? (3) Is there any difference in *L. monocytogenes* radiation D-value between frankfurters based on meat type or product formulation?

MATERIALS AND METHODS

Frankfurters

Several frankfurter types were selected for the study. The same lot number was used for each brand of frankfurter selected. Frankfurters were purchased from local markets and refrigerated at 0 to 2C prior to use. All frankfurters were purchased prior to their "sell by" dates and used prior to their expiration dates. Only packages with low background microbial counts were used in the study. The frankfurters had an average background microflora count below two log(10), or 47.6 (±15.2) cm^2 surface area, which was determined under assay conditions. The maximal contribution of background microflora to plate counts was less than one colony forming unit (CFU) at the lowest dilution used under assay conditions.

Strains

Four isolates of *L. monocytogenes* (7644, 15313,43256,49594) were obtained

from the American Type Culture Collection (Manassas, VA). The strains were propagated on Tryptic Soy Agar (Difco Laboratories, Detroit, MI) at 37C and maintained at 0 to 2C until ready for use. Strain identity was confirmed by Gram stain followed by analysis with Gram Positive Identification (GPI) cards using the Vitek Automicrobic System (bioMerieux Vitek, Inc., Hazelwood, MO).

Bacterial Cultures

Each *L. monocytogenes* strain was cultured independently in 100 mL Tryptic Soy Broth (Difco Laboratories, Detroit, MI) in a baffled 500 mL Erlenmeyer culture flask at 37C (150 rpm) for 18 h. The cultures were then combined and the mixture pelleted by centrifugation at 1725 x *g* and a temperature of 4C. The *L. monocytogenes* cells were then concentrated ten-fold by resuspension in 40 mL of Butterfield's Phosphate Buffer (BPB) (Applied Research Institute, Newtown, CT).

Inoculation and Packaging

Individual packages of frankfurters (eight to ten franks per package) were opened aseptically and individual frankfurters placed in No. 400 Stomacher bags (Tekmar, Co., Cincinnati, OH). The individual frankfurters were then surface-inoculated with 100 µL ($\approx 3.5 \times 10^{10}$ CFU), or $\approx 3.5 \times 10^{8}$ CFU per cm^2, of the concentrated *L. monocytogenes* cocktail. The frankfurters were vacuum-packed to 0.23 mmHg using a Multi-Vac Model A300 packager (Multi-Vac, Kansas City, MO) and stored at 0 to 2C until irradiation.

Gamma Irradiation

A Lockheed Georgia Company self-contained ^{137}Cs gamma irradiation source was used for all exposures. The radiation source consisted of 23 individually sealed source pencils placed in an annular array. The 22.9 cm × 63.5 cm cylindrical sample chamber was located central to the array when placed in the operating position. The inoculated vacuum-packed frankfurters were placed vertically in the sample chamber to insure uniformity of dose.

The dose rate provided by the irradiator was 0.103 kGy/min. The temperature during irradiation was maintained at 4.0(±1.0)C by the gas phase of a liquid nitrogen source which was introduced directly into the top of the sample chamber. The temperature was monitored during the entire irradiation process using two thermocouples placed 1 to 2 cm adjacent to the samples. The dose delivered was verified by use of 5 mm alanine pellet dosimeters, which were then measured using a Brucker EMS 104 EPR Analyzer. Radiation doses used were 0, 0.5, 1.0, 1.5, 2.0 and 2.5 kGy.

Plate Counts

The frankfurters were assayed for CFU's by standard pour-plate procedures. Approximately 100 mL of sterile BPB was added to a No. 400 Stomacher bag that contained an inoculated frankfurter, and the sample mixed by shaking the contents approximately fifty times. The solution was then serially diluted in BPB, using tenfold dilutions, and one mL of diluted sample pour plated using Tryptic Soy Agar medium. Three samples were plated per dilution. The plates were then incubated for 48 to 72 h at 37C prior to scoring.

D-Values

D-value is defined as the radiation dose required to produce a 90% reduction in viable organisms. The average (N) CFU/plate of an irradiated sample was divided by the average CFU/plate of the untreated control (N_o) to produce a survivor ratio (N/N_o). The D-value was then determined by calculating the reciprocal of the slope provided by the (N/No) ratios. Each experiment was conducted independently three times. The 0 through 2.0 kGy doses were used for determination of D-value. Data from the 2.5 kGy dose was excluded to eliminate possible shoulder effects, which could interfere with D-value determinations.

Statistical Analysis

Statistical analysis was completed using SAS/STAT Version 6.12 (SAS Institute, Cary, NC). Population reduction data were analyzed by analysis of variance using the general linear model procedure of the SAS statistical package (Freund *et al.* 1986; SAS Institute 1987). Comparison of regressions was performed by analysis of covariance (Thayer *et al.* 1995).

RESULTS

The frankfurter types selected for this study consisted of two brands of all beef frankfurters, three brands of poultry (mechanically deboned turkey or chicken meat) frankfurters, and two brands of mixed meat frankfurters. Mixed meat frankfurters consisted of beef, pork, and poultry mixtures, while poultry franks consisted of either turkey or a turkey and chicken mixture. The average weight per frankfurter for each brand ranged from 45 g to 57 g while the surface area ranged from 89.9 cm^2 to 99.9 cm^2 with a mean of 94.5(±1.27) cm^2. Variations in product formulation (excluding meat type, phosphates, salt and sodium nitrite) included additives such as dextrose, sucrose, maltose, high fructose corn syrup, starch, phosphates, erythorbate, lactate, ascorbic acid, soy protein concentrate, yeast lysate, spices and liquid smoke (Table 1). Frankfurter fat levels ranged from < 1% (w/w)

TABLE 1.
GAMMA RADIATION D-VALUES AND R^2 VALUES FOR SURFACE INOCULATED *L. MONOCYTOGENES* ON FRANKFURTERS

	R^2	D-value (± SE)[1]
Beef frank #1	0.98	0.52 (±0.09)
Beef frank #2	0.92	0.52 (±0.09)
Mixed meat frank #1	0.95	0.71 (±0.09)
Mixed meat frank #2	0.97	0.71 (±0.07)
Poultry frank #1	0.95	0.49 (±0.12)
Poultry frank #2	0.95	0.70 (±0.09)
Poultry frank #3	0.94	0.64 (±0.11)
Mean value	0.95	0.61 (±0.06)

[1] Values are the result of three independent experiments (n = 3), with the exception of Beef Frank #1 (n = 2). The dose rate was 0.103 kGy/min. D-values were calculated using the 0 through 2.0 kGy doses.

to 30.3% (w/w) with a mean value of 20.1 (±3.96)%. Sugar contents ranged from zero percent (w/w) to 6.0% (w/w) with a mean value of 3.26(±0.86)%.

Radiation D-values for *L. monocytogenes*, which was surface inoculated onto the seven commercially available brands of frankfurters, are shown in Table 1. The D-values of *L. monocytogenes* ranged from 0.49 to 0.71 kGy, while the average radiation D-value for all frankfurter types was 0.61(±0.06) kGy. The radiation doses used for D-value determination were zero through 2.0 kGy. Shoulder effects were observed starting at the 2.5 kGy dose. Results of analysis of covariance for pair-wise comparison of D-values ($\alpha = 0.01$) are shown in Table 2. Overall, there were nine instances of statistically significant differences in gamma radiation D-value between the frankfurter types ($\alpha = 0.01$).

Ordering of the radiation D-values based on frankfurter brand was Poultry Frank #1 < Beef Frank #1 ≈Beef Frank #2 < Poultry Frank #3 < Poultry Frank #2 ≈Mixed Meat Frank #2 < Mixed Meat Frank #1. The survival curve for all gamma irradiated *L. monocytogenes* is shown in Fig. 1. Comparison of D-values based on meat type indicated a statistically significant difference ($P < 0.01$) between the D-value of *L. monocytogenes* inoculated onto beef franks (0.52±0.16 kGy) as opposed to that of mixed meat franks (0.71±0.09 kGy). There was no difference in D-values for *L. monocytogenes* inoculated onto poultry franks (0.64 ±0.17 kGy) data versus beef frank or mixed meat for the pooled when the data was grouped by meat type.

DISCUSSION

Frankfurter is a generic term for a preformulated, batter-type, cured and cooked sausage which may consist of various meats or meat mixtures including (but not

TABLE 2.
PAIR-WISE COMPARISONS OF *L. MONOCYTOGENES* RADIATION D-VALUES[1]

	Beef Frank #1	Beef Frank #2	Poultry Frank #1	Poultry Frank #2	Poultry Frank #3	Mixed Meat Frank #1	Mixed Meat Frank #2
Beef Frank #1	X	X	X	X	X	X	X
Beef Frank #2	NS	X	X	X	X	X	X
Poultry Frank #1	NS	NS	X	X	X	X	X
Poultry Frank #2	$P < 0.01$	$P < 0.01$	$P < 0.01$	X	X	X	X
Poultry Frank #3	NS	NS	$P < 0.01$	NS	X	X	X
Mixed Meat Frank #1	$P < 0.01$	$P < 0.01$	$P < 0.01$	NS	NS	X	X
Mixed Meat Frank #2	$P < 0.01$	NS	$P < 0.01$	NS	NS	NS	X

Comparison performed using Analysis of Covariance ($\alpha = 0.01$). NS = Not Significant

The D-values of *L. monocytogenes* obtained from surface inoculated frankfurters, 0.49 to 0.71 kGy, were consistent with those found in other studies. D-values ranging from 0.57 to 0.61 kGy were found on refrigerated and frozen ground beef, with a four log(10) reduction in the number of *L. monocytogenes* at a dose of 2.5 kGy (Monk *et al.* 1994). Patterson (1989) found D-values for *L. monocytogenes* ranging from 0.42 to 0.55 kGy on poultry meat. Thayer and Boyd (1995) obtained D-values for *L. monocytogenes* on ground beef ranging from 0.45 kGy at an irradiation temperature of 5C to a D-value of 1.21 kGy at -20C. The D-value of *L. monocytogenes* on raw turkey breast meat was found to be 0.56 kGy, while the D-value on cooked turkey breast meat was found to be 0.69 kGy (Thayer *et al.* 1998). Shamsuzzman *et al.* (1992) found D-values of 0.59 and 0.68 kGy for *L. monocytogenes* inoculated onto uncooked and cooked chicken breast meat.

While the gamma radiation D-values for *L. monocytogenes* were consistent with those found for other meat products, the statistically significant differences in D-values between frankfurter brands should be noted. If a five log(10) reduction of *L. monocytogenes* contaminants were desired from the cold pasteurization process the radiation dose could vary from 2.45 kGy for Poultry Frank #1 (D-value of 0.49 kGy) to 3.55 kGy to Mixed Meat Frankfurter # 2 (D-value of 0.71 kGy). Mixed Meat Frankfurter #2 would then require a processing (irradiation) time approximately 31% greater than Poultry Frank #1.

These results suggest that "all meat" frankfurters may require a smaller radiation dose to achieve a five log(10) reduction of *L. monocytogenes* contaminants than frankfurters which contain nonmeat additives. Both brands of mixed meat frankfurters and Poultry Frank #3, which produced the highest D-values for the surface inoculated *L. monocytogenes*, contained either soy flour or soy protein concentrate. Those additives were not in the all beef frankfurters or Poultry Frankfurter #1, and produced the lowest D-values for surface-inoculated *L. monocytogenes*. The affect of additives, either in the frankfurter emulsion or applied to the product surface prior to packaging, on the radiation resistance of food-borne pathogens have yet to be determined and will be the subject of future work.

Carefully controlled studies have been conducted which determined the effect of gamma irradiation on the organoleptic qualities of frankfurters. Sensory studies on vacuum packaged frankfurters irradiated to doses of 8.0 and 30 kGy, at temperatures of -35 and -51C, varied with result (Terrell *et al.* 1981a,b,c). In those studies a radiation dose of 8.0 kGy produced undesirable sensory traits in 3 of 18 categories while frankfurters irradiated at a dose of 32 kGy were scored as being less palatable in 8 of 18 categories (Terrell *et al.* 1981a,b,c). Sensory studies conducted with vacuum packaged turkey frankfurters irradiated to doses of 0, 5.0, and 10.0 kGy, at temperatures of 2C and -30C, indicated it was possible to obtain product which was not significantly different than nonirradiated frankfurters (Barbut *et al.* 1988). Each of the studies indicated the feasibility of utilizing low

dose gamma irradiation (< 5.0 kGy) for the elimination of food-borne pathogens while maintaining acceptable product sensory attributes of the frankfurters. The higher radiation dose of 3.55 kGy required to produce a five log reduction in *L. monocytogenes* surface inoculated onto Mixed Meat Frankfurter #1 is well below the 5.0 kGy dose used by Barbut *et al.* (1988) to produce organoleptically acceptable turkey frankfurters. The dose of 3.55 kGy is also less than half the radiation dose of 8.0 kGy which produced three undesirable sensory attributes in the beef frankfurters tested by Terrell *et al.* (1981a,b,c.).

In conclusion, individual product formulation should be considered when using ionizing radiation used to eliminate *L. monocytogenes* from ready-to-eat meat products such as frankfurters. Based on data previous studies it should be possible to produce organoleptically acceptable frankfurters at the doses of ionizing radiation required to produce a five log(10) reduction of possible *L. monocytogenes* contaminants.

ACKNOWLEDGMENTS

We would like to thank L. Melenski and K. Striny for technical assistance and Dr. John Philips for assistance with statistical analysis.

REFERENCES

BARBUT, S., MAURER, A.J. and THAYER, D.W. 1988. Research Note: Irradiation dose and temperature affect on the sensory properties of turkey frankfurters. Poultry Sci. *67*, 1797-1800.

BARNES, R., ARCHER, P., STRACK, J. and ISTRE, G.R. 1989. Epidemiological notes and reports: Listeriosis associated with consumption of turkey franks. Morbid. Mortal. Weekly Rep. *38*, 268-269.

Centers for Disease Control. 1989. Listeriosis associated with consumption of turkey franks. Morbid. Mortal. Weekly Rep. *38*, 267.

FARBER, J.M., WARBURTON, D.W., GOUR, L. and MILLING, M. 1990. Microbiological quality of foods packaged under modified atmospheres. Food Microbiol. *7*, 327-334.

FREUND, R.J., LITTLE, R.C. and SPECTOR, P.C. 1986. SAS System for Linear Models, SAS Institute, Cary, NC.

FU, A., SEBRANEK, J.G. and MURANO, E.A. 1995. Survival of *Listeria monocytogenes* and *Salmonella typhimurium* and quality attributes of cooked pork chops and cured ham after irradiation. J. Food Sci. *60*, 1001-1005, 8.

GLASS, K.A. and DOYLE, M.P. 1989. Fate of *Listeria monocytogenes* in processed meat products during refrigerated storage. Appl. Environ. Microbiol. *55*, 1565-1569.

GRAU, F.H. and VANDERLINDE, P.B. 1992. Occurrence, numbers, and growth of *Listeria monocytogenes* on some vacuum-packaged processed meats. J. Food Prot. *55*, 4-7.

HUDSON, J.A. and MOTT, S.J. 1993. Growth of *Listeria monocytogenes*, *Aeromonas hydrophila* and *Yersinia enterocolitica* on cooked beef under refrigeration and mild temperature abuse. Food Microbiol. *10*, 429-437.

HUDSON, J.A., MOTT, S.J. and PENNEY, N. 1994. Growth of *Listeria monocytogenes*, *Aeromonas hydrophila*, and *Yersinia enterocolitica* on vacuum and carbon dioxide controlled-atmosphere-packaged sliced roast beef. J. Food. Prot. *57*, 204-208.

KAMAT, A. and THOMAS, P. 1998. Radiation inactivation of some food-borne pathogens in fish as influenced by fat levels. J. Appl. Microbiol. *84*, 478-484.

KERR, K.G., ROTAWA, N.A., HAWKEY, P.M. and LACEY, R.W. 1990. Incidence of *Listeria* spp. in pre-cooked, chilled chicken products as determined by culture and enzyme-linked immunoassay (ELISA). J. Food Prot. *53*, 606-607.

MANU-TAWIAH, W., MYERS, D.J., OLSON, D.G. and MOLINS, R.A. 1993. Survival and growth of *Listeria monocytogenes* and *Yersinia enterocolitica* in pork chops under modified gas atmospheres. J. Food Sci. *58*, 475-479.

MONK, J.D., CLAVERO, R.S., BEUCHAT, L.R., DOYLE, M.P. and BRACKETT, R.E. 1995. Irradiation inactivation of *Listeria monocytogenes* and *Staphylococcus aureus* in low and high fat frozen and refrigerated ground beef. J. Food. Prot. *51*, 969-974.

NICKELSON, N. and SCHMIDT, C. 1999. Taking the hysteria out of *Listeria*: The mechanics of *Listeria* and strategies to find it. Food Qual. *6*, 28-34.

OCKERMAN, H.W. 1989. In *Sausage and Processed Meat Formulations*. 1st Ed., pp. 169-193, Van Nostrand Reinhold, New York.

PATTERSON, M.F. 1989. Sensitivity of *Listeria monocytogenes* to irradiation on poultry meat and in phosphate-buffered saline. Lett. Appl. Microbiol. *8*, 181-184.

PATTERSON, M.F., DAMOGLOU, A.P. and BUICK, R.K. 1993. Effects of irradiation dose and storage temperature on the growth of *Listeria monocytogenes* on poultry meat. Food Microbiol. *10*, 197-203.

RUST, R.E. 1976. Sausage and Processed Meats Manufacturing. AMI Center for Continuing Education. pp. 89-91, American Meat Institute.

SAS Institute, Inc. 1987. SAS/STAT^c Guide for Personal Computers, Version 6 Edition, SAS Institute, Cary, NC.

SCHWARTZ, B. *et al.* 1988. Association of sporadic Listeriosis with consumption of uncooked hot dogs and undercooked chicken. Lancet ii *8614*, 779-782.

SHAMSUZZAMAN, K., CHUAQUI-OFFERMANS, L., LUCHT, T., MCDOUGALL, T.M. and BORSA, J. 1992. Microbiological and other characteristics of chicken breast meat following electron-beam and sous-vide treatments. J. Food. Prot. *55*, 528-533.

SMITH, L.T. 1996. Role of osmolytes in adaptation of osmotically stressed and chill stressed *Listeria monocytogenes* grown in liquid media and on processed meat surfaces. Appl. Environ. Microbiol. *62*, 3088-3093.

TERRELL, R.N., HEILIGMAN, F., SMITH, G.C., WIERBICKI, E. and CARPENTER, Z.L. 1981a. Effects of sodium nitrite, sodium nitrate, and DL Alpha-Tocopherol on properties of irradiated frankfurters. J. Food Prot. *44*, 414-417, 421.

TERRELL, R.N., SMITH, G.C., HEILIGMAN, E. and CARPENTER, Z.L. 1981b. Cooked product temperature and curing ingredients affect properties of irradiated frankfurters. J. Food Prot. *44*, 215-219.

TERRELL, R.N., SWASDEE, R.L., SMITH, G.C., HEILIGMAN, E. and CARPENTER, Z.L. 1981c. Irradiation of frankfurters-effects on sensory properties. Food Dev. *15*, 62-64, 66-67.

THAYER, D.W. and BOYD, G. 1995. Radiation sensitivity of *Listeria monocytogenes* on beef as affected by temperature. J. Food Sci. *60*, 237-240.

THAYER, D.W., BOYD, G., FOX, J.B. and LAKRITZ, L. 1995. Effects of NaCl, sucrose, and water content on the survival of *Salmonella typhimurium* on irradiated pork and chicken. J. Food Prot. *58*, 490-496.

THAYER, D.W., BOYD, G., FOX, J.B. and LAKRITZ, L. 1997. Elimination by gamma irradiation of *Salmonella* spp. and strains of *Staphylococcus aureus* inoculated in bison, ostrich, alligator, and caiman meat. J. Food Prot. *60*, 756-760.

THAYER, D.W., BOYD, G., FOX, J., LAKRITZ, L. and HAMPSON, J.W. 1995. Variations in radiation sensitivity of food-borne pathogens associated with the suspending meat. J. Food Sci. *60*, 63-67.

THAYER, D.W., BOYD, G., KIM, A., FOX, J.B. and FARRELL, H. 1998. Fate of gamma-irradiated *Listeria monocytogenes* on raw or cooked turkey breast meat. J. Food. Prot. *61*, 979-987.

USDA. 1989. Revised policy for controlling *Listeria monocytogenes*. Fed. Reg. *54*(98), 22345-22346. Food Safety and Inspection Service, U.S. Dept. of Agriculture, Washington, DC.

WANG, C. and MURIANA, P.M. 1994. Incidence of *Listeria monocytogenes* in packages of retail franks. J. Food Prot. *57*, 382-386.

WEHR, M. 1987. *Listeria monocytogenes* - A current dilemma. J. Assoc. Off. Anal. Chem. *70*, 769-772.

WENGER, J.D. *et al.* 1990. *Listeria monocytogenes* contamination of turkey franks: Evaluation of a production facility. J. Food Prot. *53*, 1015-1019.